BACTERIOCINS AND BACTERIOCIN-LIKE SUBSTANCES

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I. Introduction

Jacob et al. (28) defined the notion of bacteriocins as follows. Bacteriocins are protein-like substances the biosynthesis of which is associated with a lethal consequence for the producing organisms. The action of a bacteriocin is restricted to only a limited number of related species, and some act only on certain strains of the same species which produce them. The action of bacteriocins is conditioned by the presence of specific receptors.

Elaboration of bacteriocins or bacteriocin-like substances has been described in a number of species belonging to various families of the class *Schizomycetes*. The very narrow range of their antibacterial spectra sharply delineates bacteriocins from antibiotics.

II. Colicin

A. General characteristics of colicin. Colicins are produced by different species of the family *Enterobacteriaceae*. A number of recent reviews (10, 11, 31) give a full description of their features, and it seems needless to deal with them in detail here. After summarizing their main characteristics, only recent data which are not included in the excellent review of Fredericq (10) will be treated.

Colicins are produced by individual strains of *Escherichia coli* and different *Salmonella* and *Shigella* types. Colicins are grouped by their bactericidal specificity. On this basis, nearly 20 different colicins are described which are designated in alphabetic order. The bactericidal action of each colicin is limited to species of enterobacteriaceae. Some of them act on only a few strains, whereas others may have a somewhat wider range of action. As a general rule, strains producing colicin are immune to their own colicin; however, an exceptional case is known where the bacteria are sensitive to the same colicin which is produced by them (45). Colicins are antigenic in accordance with their protein character. When injected into rabbits they give rise to antibodies that both neutralize the bactericidal effect and give a specific precipitate with colicin.

Mutants resistant to colicin appear in sensitive populations. Colicinogeny is a permanent hereditary character which is not lost by serial transfer

[1] A part of this review was presented in "Symposium on Mechanisms of Bactericidal Action," 60th Annual Meeting of The Society of American Bacteriologists, Philadelphia, 1960. The author is indebted to Bernard D. Davis for reading and correcting the manuscript.

of strains. In some cases, noncolicinogenic mutants were isolated from strains producing colicin (3).

Colicin is adsorbed by specific receptors situated on the surface of sensitive bacteria. A resistant mutant appears to lack this specific receptor. A single receptor may be common to colicin and to a certain bacteriophage. Thus the receptor of colicin K and that of phage T6 appear to be identical or closely related, since the development of resistance to phage T6 is associated with a simultaneous loss of sensitivity to colicin K. Still, there is a fundamental difference between phage and colicin; colicins are inert proteins not able to multiply in sensitive bacteria. Furthermore colicin kills bacteria without lysis.

Fredericq (7) assumes that the relationship between colicins and phages exists only on the level of the genes which determine their production. The products, colicin and phage, are materially distinct.

B. Elaboration of colicin. According to Jacob et al. (28) one of the criteria of colicins is their lethal biosynthesis: that is, the production of colicin involves death of the bacterium. Experimental data on this issue are still in several respects incomplete. One of the most demonstrative observations appeared to be the inducibility of *E. coli* strain ML, which lyses and releases colicin after ultraviolet irradiation (30). However, it was found later that this strain is not only colicinogenic but also lysogenic, carrying a number of inducible prophages; and the lysis is not due to the colicinogeny but to the lysogeny (33). Nevertheless, ultraviolet irradiation can enhance colicin production without lysis (9). An increased colicin production was also observed when the much studied *E. coli* strain B was irradiated (6).

Ozeki and his associates (44) carried out a detailed investigation with a *Salmonella typhimurium* strain which was artificially made colicinogenic for E2 colicin to study the kinetics of colicin production. A light suspension of this strain was mixed with sensitive indicator cells and incorporated in a soft agar layer. About 1% of the colicinogenic bacteria caused a tiny clear spot in the indicator "lawn." The number of clear spots was much increased when the colicinogenic bacteria were irradiated with a light dose of ultraviolet light before plating. In micromanipulative isolation about 50% of the individual bacteria released colicin, which is a higher proportion than that found by plating. It is striking that clear spots in the indicator lawn were also formed even when the colicinogenic cells had been killed with chloroform vapor before inoculation, or when streptomycin-sensitive colicinogenic bacteria were plated in streptomycin agar with a streptomycin-resistant indicator. However, the number of clear spots was 10- to 100-fold less than in the absence of streptomycin. This means that there was, previous to the plating, enough colicin in the organisms to produce a clear spot. It was found, furthermore, that all cells of an irradiated culture which release colicin are nonviable. Similar observations were made with other strains of *S. typhimurium* producing other colicins. These careful studies confirmed the lethal consequence of the biosynthesis of colicins.

C. Genetics of colicin production. Fredericq (8, 10) observed the transfer of colicinogeny in *E. coli* strains in $F^+ \times F^-$ systems. The transfer appears to be a consequence of bacterial conjugation. When F^+ *col*$^+$ is the donor, the colicinogeny is transferred to F^- *col*$^-$ recipient; and the transfer is independent of that of other markers. It was striking, however, that the noncolicinogenic character cannot be transferred from the donor parents to the recombinants. Fredericq concluded from this asymmetry that colicinogeny is not governed by chromosomal genes but rather is under the control of cytoplasmic determinants. These determinants are transferred by a direct contact between bacteria of opposite mating type. The production of only certain colicins could be carried over this way; some colicin factors, like that of colicin I, could not be transmitted.

Alföldi et al. (1, 2) found similar results when they used Hfr instead of F^+ donors. Hfr *col*$^+$ $\times$ F^- *col*$^-$ crosses yielded *col*$^+$ recombinants; but crosses of Hfr *col*$^-$ $\times$ F^- *col*$^+$ did not yield a *col*$^-$ recombinant. Failure to isolate noncolicinogenic recombinants was found to be due to a lethal effect on such recombinants (*zygose létale*). Furthermore, in crosses with individual Hfr mutants, in which the sequences of introduced markers were different, the frequency of transfer of colicinogeny was characteristic for each donor and the proportion of surviving recombinants was in inverse ratio to the transfer of colicinogeny. It was concluded from these results that the genetic determinants of colicin production may also exist in chromosomes.

Stocker (46) found a fundamental difference in the transfer of various strains of colicinogeny in *S. typhimurium*. Colicin I is transmitted to another strain by cell contact, whereas colicin E2 is not transferable in this way. When a strain with colicin E2 is made colicinogenic for colicin I, both characters are transmissible. Thus the colicin I agent is reminiscent of the F agent in *E. coli*. A new horizon is opened in the genetics of enterobacteriaceae by the discovery that certain colicinogenic factors are able to function as a mediator of chromosomal genes in bacterial crosses (5, 43).

Jacob et al. (29) class the colicinogenic factor among the episomes. The concept of episome defines a class of genetic element which may exist in two alternative states. They either may be replicated autonomously, quite independently of the chromosomal material, or they may be integrated into the bacterial chromosome and their replication be strictly associated with that of chromosomal material. In addition, as already mentioned, a colicinogenic factor may function as a fertility factor in transmitting chromosomal genes.

Phage-mediated transduction of colicinogeny has been also described (12). Phage material of PLT22, obtained by propagating phage on a colicinogenic *S. typhimurium* strain, was used. The colicinogenic factor was transduced to a noncolicinogenic acceptor quite independently of other chromosome markers. This finding further supports the suggestion that the colicinogenic factor may be integrated into the chromosomal material.

D. Biochemistry of colicin. A number of attempts have been made to purify and describe the biochemical features of individual colicins. The earlier investigations have recently been summarized carefully by Cocito and Vandermeulen-Cocito (6). The sensitivity of colicins tested so far to proteolytic enzymes indicates their protein or peptide character.

A detailed study of colicin K by Goebel, Barry, and Shedlovsky (13) is of particular interest. A high production of colicin was found in a culture of *E. coli* K235 when the pH of the growing culture was kept at a constant level. Chemical fractionation yielded a highly purified preparation proved to be identical with the immunospecific lipocarbohydrate-protein component of the strain. Colicin K activity was intimately associated with this substance, which indicates that colicin K activity is an inherent property of the O antigen of this particular strain. Crystalline trypsin destroyed the colicin activity of the preparation. By treatment with 90% phenol the complex is dissociated, yielding a protein constituent with colicin activity. This protein has a very potent antibacterial action and gives rise to antibody formation when injected into rabbits. The sera precipitate colicin and neutralize its bactericidal action (14).

A detailed immunological study (3) of a purified colicin K preparation revealed a number of interesting data concerning the immunobiological activity of colicin K. The complex of colicin with O antigen elicits the formation of antibodies with diverse serological activity. One of the antibodies is capable of neutralizing the bactericidal action of colicin without precipitating it, whereas the other antibody component precipitates colicin without abolishing its bactericidal action. The serological tests could not support the assumption (34) that colicin K and T6 phage are related.

As to the site of colicin K production, it has been assumed that the protein is synthesized inside the cytoplasmic membrane and combines with somatic O antigen in the cytoplasmic membrane, where O antigen is synthesized (36).

A lipocarbohydrate-protein complex of high colicin activity was also isolated by Nüske et al. (42) from another colicinogenic *E. coli* strain SG710, the colicin of which was not classed among the known colicins. The colicin produced by this strain was readily adsorbed by glass powder and could easily be eluted with dilute sodium carbonate solution. The purified colicin appeared to be uniform in paper electrophoresis. The amino acid composition, and inactivation of the bactericidal action by proteolytic enzymes, indicated that a protein bears the colicin action of the complex.

III. Pyocin

A. General characteristics of pyocin. Jacob described (27) a new antibacterial principle produced by one strain of *Pseudomonas aeruginosa* which proved not to be identical with other antibiotics such as pyocyanin. This substance, named pyocin, is active on other strains of *P. aeruginosa*. Synthesis of pyocin is induced by a light dose of ultraviolet irradiation of the

culture; during subsequent incubation mass lysis of bacteria ensues with liberation of pyocin into medium.

Hamon (15) found 10 *P. aeruginosa* strains out of 15 examined to produce pyocin. Most of these strains were also found to be lysogenic. Various strains of *Salmonella*, *Shigella*, and *Proteus* appeared generally to be resistant to pyocin, but R mutants of these species were highly sensitive.

None of the pyocins were studied in detail. They do not pass cellophane, but they diffuse in agar gel. Their sensitivity to heat and proteolytic enzymes varies accordingly to individual strains which produce them.

B. Mode of action of pyocin. Pyocin is fixed by specific receptors of sensitive bacteria. When various dilutions of pyocin were added to identical portions of a bacteria suspension a rapid drop in the number of colony formers appeared. In about 5 min the velocity of killing strikingly diminished, and at the higher dilutions of pyocin the number of living bacteria remained nearly constant. It was concluded (27) that to kill each bacterium a certain amount of pyocin was needed, which could be considered a lethal unit.

Adsorption of pyocin on bacteria did not have an instantaneous killing effect on the organisms, for respiration of the bacterial suspension decreased gradually for a considerable period of time. Nevertheless, bacteria that have adsorbed pyocin do not multiply. It appears that pyocin exerts an irreversible bacteriostatic effect on bacteria which leads to the death of the cells.

IV. Megacin

A. Iso-antagonistic effect of Bacillus megaterium strains. It was found (18, 25) that some strains of *Bacillus megaterium* exhibit an iso-antagonistic effect on most of the *B. megaterium* strains tested. This effect was especially clearly expressed when certain phage-resistant mutants (24) were used as indicator strains. These strains had the advantage of excluding interference by lysogeny, which also occurs in a proportion of *B. megaterium* strains.

In detecting the iso-antagonistic effect of *B. megaterium* strains, agar plates were overlaid with a soft agar layer containing indicator bacteria. A drop of the culture to be tested was put on the surface of two such indicator plates, one of which was then irradiated with a light dose of ultraviolet light before incubation. It was found that the iso-antagonistic effect of *B. megaterium* strains was usually very much increased by ultraviolet irradiation, and that some positive strains showed no detectable effect without this irradiation (Fig. 1).

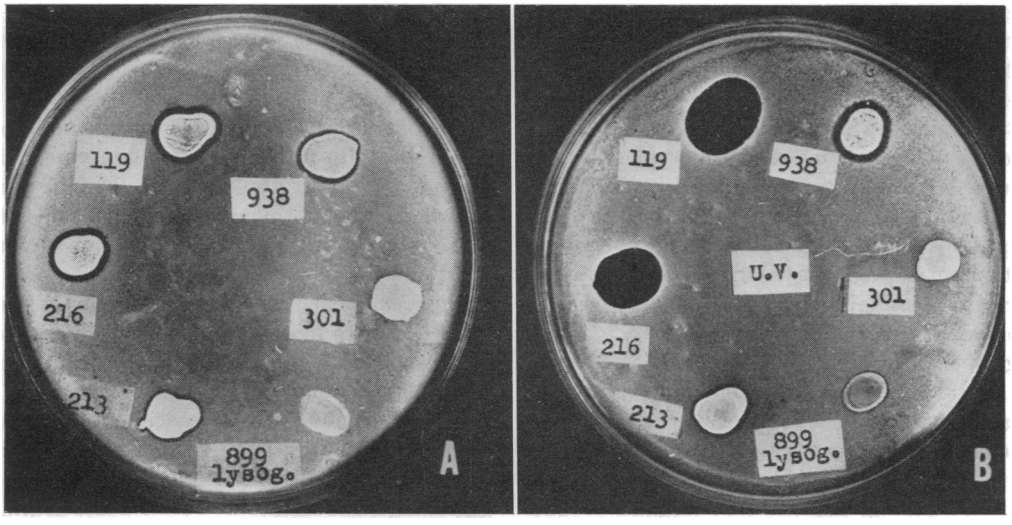

FIG. 1. *Cultures of different Bacillus megaterium strains dropped on plates seeded with a phage-sensitive strain; a, not irradiated; b, irradiated before incubation. Strains 119 and 216 are fully inducible. There is only a partial induction in the case of lysogenic strain 899/1/. Strain 301 is a nonmegacinogenic strain.*

Two hundred strains of *B. megaterium* isolated from soil samples were tested in this way (25). Nearly half the strains exhibited an isoantagonistic effect. Nineteen strains giving a maximal effect lysed totally after ultraviolet irradiation. The phenomenon is somewhat similar to lysogeny, but all attempts failed to transfer the lytic principle to phage-sensitive bacteria. The bactericidal action of lysed bacteria induced by ultraviolet irradiation is due to a soluble material which has been classed with bacteriocins and named *megacin* (18).

B. Kinetics of megacin formation. The best studied megacinogenic strain, 216, proved to be highly inducible with ultraviolet irradiation, and a powerful producer of megacin. Lysates from this strain had antibacterial titers up to 1/40,000, representing the highest dilution of which a drop gives visible clearing on an indicator plate. When strain 216 is grown in certain appropriate media the usual exponential curve can be seen, and even after full growth no megacin, or only traces, can be detected in the supernatant. This means that megacin is not excreted normally by growing bacteria; its appearance is always associated with lysis. With some *B. megaterium* strains, such lysis can occur spontaneously in certain media, but in other media the action of an inducing agent, such as ultraviolet light, is required (25). Ultraviolet irradiation of an exponentially growing culture of a megacinogenic strain initiates a series of cytological events which finally lead to total lysis of the bacteria associated with liberation of megacin (19). Twenty to thirty minutes after irradiation and reincubation the bacteria become elongated and their division gradually ceases. When bacteria are stained in this phase of growth with a special cell-wall staining technique, not only the cell wall but some part of the cytoplasm also adsorbs the dye. This indicates an increased permeability of the cell envelope. The tinctorial changes are associated with morphological changes in the bacteria which lead finally to a total lysis by 90 to 100 min after ultraviolet irradiation. Only a few intact or partially lysed bacteria are seen among the debris and remnants of the lysed cells.

These findings indicate that megacin production is not associated with the normal multiplication of cells but is a lethal biosynthesis elicited by ultraviolet irradiation. It was estimated that the release of megacin begins in about two generation times after induction, and is completed within another generation time (19).

C. Antibacterial spectrum of megacin (20, 41). Lysates obtained from various megacinogenic strains are effective on all *B. megaterium* strains tested so far. Hundreds of *B. megaterium* strains tested were found to be sensitive to a different degree to megacin. It is important to note that even strains which produce megacin are sensitive to their own megacin. Among many gram-positive and gram-negative species other than *B. megaterium*, only some pigment-forming aerococcus strains, tentatively identified as *Micrococcus aurantiacus*, and *Micrococcus cinnabareus*, were found to be sensitive to megacin; and a very limited sensitivity was displayed by some *Bacillus anthracis* strains. Some atypical *Bacillus subtilis* strains were also found to be sensitive to megacin to a low degree.

There is a striking difference between megacin and egg white lysozyme sensitivity. *Sarcina flava*, which is highly sensitive to lysozyme, is not at all sensitive to megacin.

D. Some characteristics of megacin. Megacin of strain 216 has been characterized biochemically to some extent with concentrated preparations of high potency obtained from lysates in a synthetic medium (22). Concentrates contained about 3,000 units of megacin per μg of nitrogen.

Megacin does not dialyze through cellophane. It is highly sensitive to alkaline buffer, but in a phosphate buffer of pH 5.6 to 7.0 it is stable for weeks in a refrigerator. Megacin is rapidly inactivated at 80 C.

The protein nature of megacin is indicated by its sensitivity to authentic samples of proteolytic enzymes. Twice crystallized pepsin rapidly inactivated megacin, chymotrypsin had a moderate effect, whereas twice crystallized trypsin had no effect (26).

When megacin concentrates obtained from strain 216 were repeatedly injected into rabbits, increasing amounts of antibody appeared in the course of the immunization. The antisera gave a specific precipitate with megacin concentrates, but only a slight precipitate with extracts of noninduced cells of strain 216 and only a weak agglutination of such cells. The sera also neutralize in high titer the bactericidal action of megacin. In contrast, antisera to the uninduced cells gave high titer agglutination of the bacteria and no neutralization of megacin (26).

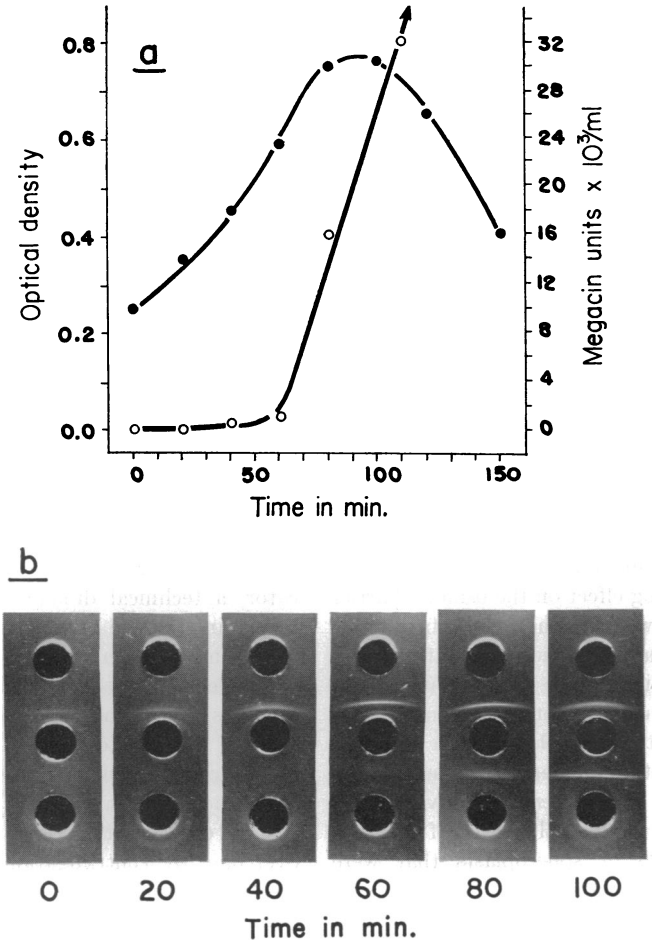

FIG. 2. *Course of formation of (a) bactericidal activity and (b) the immunospecific substance in an ultraviolet-irradiated culture of Bacillus megaterium strain 216. Ultraviolet irradiation at 0 min. The samples were lysed with crystalline lysozyme (10 μg per ml). a. Optical density of the culture (●——●); megacin titer (○——○). b. Immunospecificity of individual samples taken at intervals from the culture as shown by gel diffusion. The extracts were added into the central holes. Upper holes contained antibacterial serum, whereas the lower holes were filled with specific anti-megacin serum (from reference (26)).*

Highly specific antimegacin serum, obtained by absorption of serum with a lysozyme extract of noninduced cells of strain 216, could be used to follow the formation of megacin in an induced 216 culture (Fig. 2). The appearance of a new immunospecificity is paralleled with the degree of lysis and of the accumulation of a bactericidal substance in the culture. This observation indicates that the appearance of the protein-like megacin in the ultraviolet-irradiated culture is a consequence of a new biosynthesis which is lethal for the cells producing the material (26).

The megacin of strain 216 was isolated recently from the lysate in homogeneous form by electrophoresis and ultracentrifugation. From sedimentation velocity experiments a value of 51,000 was obtained for the molecular weight. Megacin of strain 216 appears to be a simple protein which is capable of inhibiting the growth of sensitive cells at a concentration of 10 mμg per ml (17).

Megacins of different strains have nearly identical antibacterial spectra but differ in immunospecificity. Anti-megacin serum to strain 216 was found to be ineffective in neutralizing the antibacterial action of megacin obtained from other strains. In addition, attempts to pro-

duce antibodies by immunizing rabbits with two other megacins have failed (41).

E. Mode of action of megacin. The action of megacin (22) is bactericidal. In its presence the number of colony formers decreased rapidly at 40 and 29 C. There was only a moderate decrease at 12 C and none at 0 C.

When megacin was added to growing cultures of sensitive bacteria, the turbidity gradually decreased. Phase contrast microscopy showed that the intracellular components escaped into medium, and finally only empty cell walls were seen. Megacin exhibited this effect only on living bacteria.

The increase in cell permeability was also shown by the escape of ultraviolet-absorbing material into the medium, and by the observation that megacin, like toluene, increased the activity of the enzyme β-galactosidase in intact cells harvested from a medium containing lactose.

The most striking effect on the osmotic barrier of *B. megaterium* was demonstrated when stable lysozyme protoplasts in sucrose solution were exposed to megacin. It was found that 2 to 10 units per ml of megacin obtained from various strains converted the protoplasts into a ghostlike structure. This action of megacin appears to be highly specific for *B. megaterium*. Only bacteria sensitive to the bactericidal action of megacin yielded protoplasts or spheroplasts that were destroyed by the substance.

The various antibacterial substances produced by different strains of *B. megaterium*, and inducible by ultraviolet irradiation, have an identical mode of action. This justifies denoting these substances by a common name (41).

The observations presented above indicate that megacin causes a radical change in the osmotic barrier of sensitive bacteria. Megacin apparently does not attack the cell wall but destroys the cytoplasmic membrane. The fact that the bactericidal effect of megacin is highly dependent on temperature suggests the participation of an enzyme, but it is not yet clear whether megacin is an enzyme or whether an autolytic process in the cell or protoplast is enhanced by megacin.

The failure of megacin to attack the cell wall of *B. megaterium* distinguishes its action from that of enzymes produced by phage-infected *B. megaterium* (38), or isolated from megaterium phage (39).

When cells are exposed to even a high dilution of megacin they do not absorb enough of the substance to decrease the titer of the fluid. This behavior stands in marked contrast to some lytic antibiotics such as polymyxin. In this respect the behavior is also in contrast to that of phages and of colicins.

F. Genetics of megacin production. A proportion of *B. megaterium* strains isolated from natural sources are free of either lysogeny or megacinogeny (18). The occurrence of the megacinogenic property appears to be considerably more frequent than lysogeny. A detailed study of 100 *B. megaterium* strains (40) isolated from fecal or soil samples revealed that 15 were lysogenic, 34 megacinogenic, and 19 both lysogenic and megacinogenic. Nearly one third of the strains were neither lysogenic nor megacinogenic.

Although there is no difficulty in assaying megacinogeny in lysogenic strains of *B. megaterium* by using a phage-resistant strain as indicator, a technical difficulty exists in trying to reveal an abortive phage production in megacin-producing bacteria. Abortive phage production, associated with a low yield of infective phage particles, is known among *B. megaterium* strains (32, 35). The detection of but a few infective phages could be interfered with by the presence of megacin, which kills the indicator cells at a certain concentration. This difficulty could be overcome by centrifugation of culture fluid or lysate of megacinogenic strains at 100,000 × *g*, followed by assay of the pellet with different indicator strains for the presence of phage particles. Tested in this way, 10^9 organisms of each of 14 megacinogenic strains did not yield any infective phage particle (25). One megacinogenic strain, 216, which is particularly effective in producing megacin after ultraviolet irradiation, was subjected to electron microscopy to detect the potential presence of incomplete phage structures. Neither mature phage particles nor structures reminiscent of incomplete phage were found around the cells in different stages of lysis (21). On the other hand, a well defined protein with antibacterial action was isolated from the lysate of this strain (17).

Non-megacinogenic mutants (or segregants?) were isolated from various megacinogenic strains; they did not lyse after ultraviolet irradiation and did not yield an antibacterial substance. The frequency of these non-megacinogenic mutants varied from approximately 0.3 to 10% in spores of individual strains. It is supposed that there is

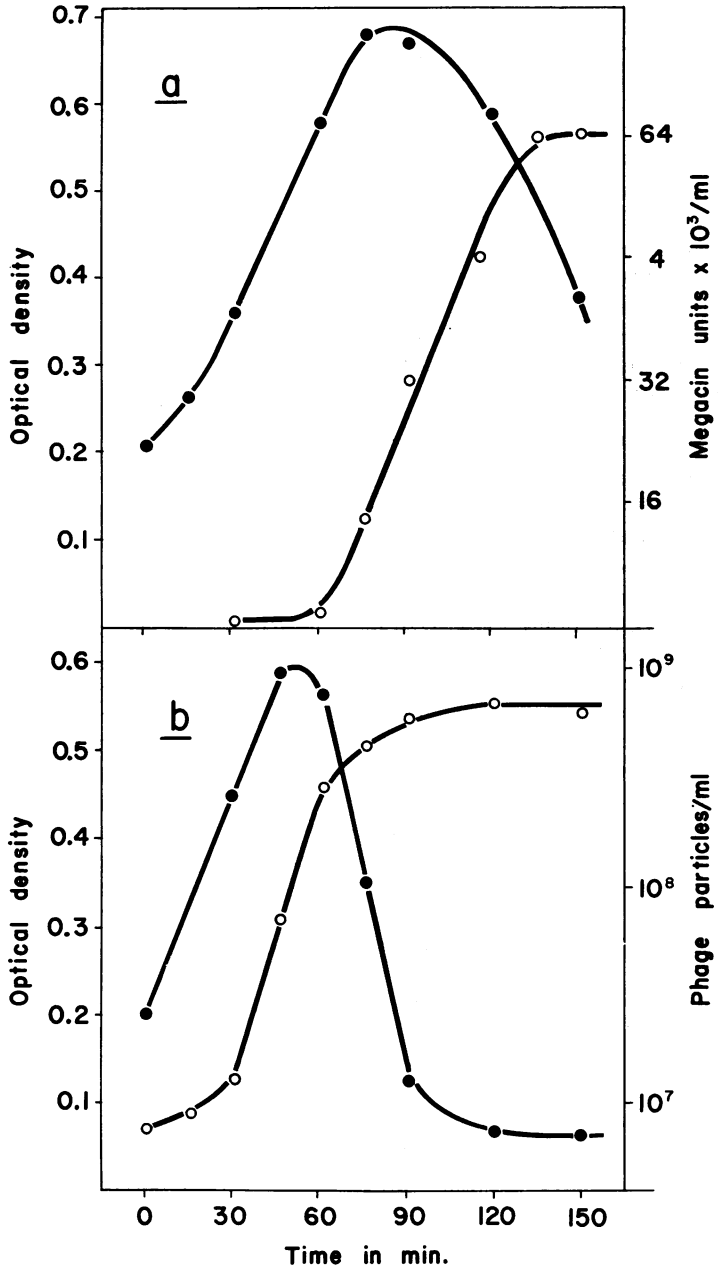

FIG. 3. *Similarity of megacinogeny and lysogeny after ultraviolet induction and reincubation under identical conditions. a. Induction of megacinogenic strain 216; b. induction of a typical lysogenic strain 899/1/ of Bacillus megaterium. Optical density* (●——●); *megacin and phage production* (○——○), *respectively.*

a factor perpetuated in megacinogenic bacteria which governs the hereditary character of megacinogeny (25).

Megacinogeny simulates lysogeny in several respects, except that no infective phage is produced (Fig. 3). Instead, a new protein is synthesized in induced cells as a consequence of a lethal biosynthesis. It calls to mind the known cases of

defective lysogeny that are lethal for the host without the release of infectious particles. In such cases, one should consider the existence of a "masked provirus" ("exprophage") which cannot be recognized since it does not yield the typical product of lysogeny. The hypothesis is advanced that megacinogeny is due to a highly defective lysogeny which results in a new protein synthesis of lethal character (23). Some attempts have been made in this laboratory to convert a typical lysogenic *B. megaterium* strain into a megacinogenic one, but without success. Whether or not megacin is a protein part of a hypothetical temperate phage awaits further evidence.

V. Other Bacteriocin-like Substances

A number of antibacterial substances acting on the same species which produce them have been described but little studied. In the following a brief summary is given of some examples.

Most strains of *Pasteurella pestis* produce an antibacterial substance inhibiting the growth of *Pasteurella pseudotuberculosis* strains (4). The protein-like substance named *pesticin* has a very narrow antibacterial spectrum, limited only to *P. pseudotuberculosis* and to a *P. pestis* strain that is not a pesticin producer. The formation of pesticin is increased after ultraviolet irradiation but is not accompanied by total lysis of bacteria. It is assumed that only a small proportion of the population produces pesticin and undergoes partial lysis (4).

The synthesis of pesticin in irradiated bacteria requires certain amino acids. Furthermore, the synthesis is inhibited by a sub-bacteriostatic concentration of chloramphenicol (16).

A heat-labile, dialyzable substance sensitive to protein denaturants was detected in some atypical strains of *Mycobacterium tuberculosis*. This bacteriocin-like substance is filtrable through a Selas filter and did not prove to be an infective phage (37).

A number of *gravis* type *Corynebacterium diphtheriae* strains exert an antibacterial effect on other strains of the same species. The substance produced is not sensitive to proteolytic enzymes. It is not active against various gram-positive cocci and a number of gram-negative species (47).

VI. Conclusion

One criterion in the definition of bacteriocins recommended by Jacob et al. (28) is the specific fixation of bacteriocins by sensitive bacteria. This feature is fulfilled by colicins and pyocins, which are both readily bound by specific cell receptors. A variety of evidence indicates that colicin synthesis, or its release from bacteria, exerts a lethal effect on the cell which produces it. This lethal aspect of colicinogeny, the protein nature of colicin, and its specific adsorption by sensitive cells, are characteristics which correspond to a prototype of bacteriocin.

The production of pyocin has been very little studied as yet, and one cannot rule out the possibility that a prophage or a defective prophage is playing a role in the induction and lysis of pyocinogenic strains. Whether pyocin production itself has a lethal consequence on cells which elaborate it, or whether the lysis of bacteria is due to the presence of a concomitant prophage, should be ascertained.

Megacin is a product of a new protein synthesis which is triggered by some inducing agent, and the synthesis of which involves the lysis of cells. Megacinogeny is perpetuated by a hereditary unit in its latent form, and the expression of its function is a lethal process for the bacteria. Megacinogeny simulates lysogeny so closely that one should consider that megacin production may be the release of immature phage components or intermediates in phage synthesis. The mode of action of megacin is not associated with the presence of specific receptors on the cell wall of sensitive bacteria; instead, the osmotic barrier (the integrity of the cytoplasmic membrane) is broken down by its action.

As to the bacteriocin-like substances elaborated by other bacterial species, our knowledge is quite incomplete. How much they correspond to the definition of bacteriocins is hard to say. Still, to name them "bacteriocin-like substances" appears to be useful to distinguish them from antibiotics in the general sense.

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